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Immunology

Delineation of the minimal hepatitis B surface antigen-specific B- and T-cell epitope contained within an anti-idiotype-derived pentadecapeptide

(epitope mimicry/crossreactivity/truncated peptides)

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ABSTRACT **A pentadecapeptide (2F10 peptide) is capable of mimicking the group-specific "a" determinant of human hepatitis B surface antigen (HBsAg) at both the B- and the T-cell level. This peptide represents a sequence on the heavy-chain hypervariable region of a monoclonal "internal image" anti-idiotype (anti-id 2F10) that has partial sequence homology to the "a" determinant epitope of HBsAg. To identify the exact location of the B- and T-cell epitopes, four truncated peptides (peptides 1–4) were synthesized. Using these truncated peptides we have identified the minimal sequence (octapeptide 3) that represents a functional B- and T-cell epitope capable of generating HBsAg-specific antibodies and T cells. This to our knowledge represents the first example of a short peptide sequence functioning as both a B- and a T-cell epitope. We have also identified another T-cell epitope (2F10 peptide 4), but this peptide fails to elicit HBsAg-specific B cells and T cells. Thus, the 2F10 pentadecapeptide is composed of two nonoverlapping, functional T-cell epitopes only one of which is HBsAg specific. Since peptide 3 represents the complementarity-determining region and peptide 4 represents the framework region of the anti-id 2F10, we conclude that an 8-aa sequence from the complementarity-determining region of anti-id 2F10 is sufficient for the molecular mimicry of HBsAg. Finally, our experiments suggest that sequences flanking the minimal immunodominant epitope exert a considerable influence on the nature of antigenic processing that occurs and the resultant T-cell reactivity elicited.**

The efficacy of viral vaccines is often measured by the presence of protective antibodies. An important requisite for the synthesis of antibodies is efficient induction of a T helper (T_h) response towards peptide sequences of viral proteins. Several laboratories are endeavoring to combine well-characterized T_h and protective B-cell epitopes to create effective synthetic peptide vaccines (1–3) for the control of viral and parasitic diseases.

Jerne's immune network theory (4) proposes that several types of anti-idiotype (anti-id) antibodies are produced during the immune response to a given antigen. A subset of these anti-id antibodies termed "internal-image" antibodies or Ab2β are predicted to mimic the molecular features of the nominal antigen (5, 6). This hypothesis is based on the concept that certain regions of the anti-id which are homologous to the immunogenic epitopes of the infectious organism functionally mimic these epitopes and induce protective immune responses (5, 6). Such anti-id antibodies have been used in experimental systems as surrogate vaccines against several bacterial, viral, and parasitic organisms (7).

Anti-id monoclonal antibody (mAb) 2F10, produced against a monoclonal id designated H3F5 (8), recognizes the protective determinant epitope on the envelope protein [hepatitis B surface antigen (HBsAg) residues S-(139–147)] of the hepatitis B virus. Mimicry of HBsAg by anti-id 2F10 is associated with a 15-aa sequence on its heavy-chain hypervariable region (9). This sequence is partially homologous with the a determinant of HBsAg. A synthetic peptide (2F10 peptide) corresponding to this sequence can duplicate the B- and T-cell-stimulatory activity of the intact anti-id (2F10) and HBsAg (9).

To understand the molecular mimicry of this peptide, we made truncated peptides of this 15-aa sequence to allow us to determine the placement of B- and T-cell epitopes within the 2F10 peptide. We have established that the C-terminal portion of the 2F10 peptide (peptide 3) contains both the T- and the B-cell epitopes necessary to generate an antibody response against HBsAg. We have also identified another, nonoverlapping T-cell epitope in the 2F10 peptide sequence, but this peptide does not function as a B-cell epitope. Further, based on our observations that 2F10 peptide requires antigenic processing and that peptides 3 and 4 do not require any further processing to elicit their T-cell responses, we predict a proteolytic cleavage site within the 2F10 peptide sequence between arginine and glycine. Cleavage at this site would result in the 2F10 peptide being processed into the minimal essential immunodominant epitope which would correspond to peptide 3, which qualitatively fulfills all the functional criteria of the 15-aa peptide and also of the native protein antigen.

MATERIALS AND METHODS

Mice. Female BALB/c mice (H-2^d) were obtained from Springville Laboratories (Springville, NY). All mice were 6–8 weeks old at the beginning of the study.

***In Vivo* and *in Vitro* Stimuli.** 2F10 is a mouse internal-image anti-id mAb which mimics the group-specific a determinant of HBsAg (8). The murine mAb 2C3 (anti-phthalate) was used as an isotype control (S. Ghosh, Indiana State University). Soluble recombinant HBsAg devoid of pre-S proteins was provided by W. F. Miller (Merck Sharp & Dohme). 2F10 peptide represents a 15-aa region of the heavy-chain hypervariable region of mAb 2F10. S peptide [S-(139–147); CTKPTDGNC] represents a part of the sequence that constitutes the a determinant on HBsAg (10). Peptides 1–4 constitute sequences which are truncated variants of the 15-aa 2F10 peptide (Fig. 1). All the peptides were synthesized by solid-phase methods (Biopolymer Core Facility, Roswell Park Cancer Institute) and were conjugated to keyhole limpet hemocyanin (KLH) with glutaraldehyde.

Induction of Anti-HBs Antibodies. BALB/c mice (five per group) were immunized i.p. with the truncated variants of the

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Abbreviations: HBsAg, hepatitis B surface antigen; mAb, monoclonal antibody; id, idiotype; CDR, complementarity-determining region; KLH, keyhole limpet hemocyanin; FR, framework region.

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2F10 peptide	^{1}AVYYCTRGYHGSSLY^{15}
Truncated peptides	
Peptide 1	^{1}AVYYCTRGYHGS^{12}
Peptide 2	^{5}CTRGYHGSSLY^{15}
Peptide 3	^{6}GYHGSSLY^{15}
Peptide 4	^{1}AVYYCTR^{7}

FIG. 1. Sequence of 2F10 peptide and its truncated variants. Superscript figures indicate the residue number in the 15-aa sequence.

2F10 peptide. The peptides coupled to KLH were administered at 100 μg per injection in complete Freund's adjuvant, incomplete Freund's adjuvant, and 0.9% NaCl at days 0, 7, and 14, respectively. Mice were bled weekly for the indicated time period, and the sera were tested for anti-HBs antibodies with an ELISA kit (AUSAB; Abbott Diagnostic Laboratories).

***In Vitro* Proliferation of Mouse Lymph Node T Cells.** Mice were immunized in the hind footpads with either 2F10 peptide or the truncated variants of 2F10 peptide coupled to KLH. The amount of immunogen and the schedule of immunization were as described for induction of anti-HBs antibodies. One week after the third injection, the mice were sacrificed and the popliteal lymph nodes were collected and teased apart, and T cells were purified by use of nylon wool (11). Enriched T cells (2.5×10^5 in 100 μl) were plated in 96-well flat-bottomed plates along with 5×10^5 irradiated syngeneic spleen cells as a source of antigen-presenting cells. Stimuli were added to the cells in triplicate cultures. The cells were then cultured for 120 hr. Proliferation, as measured by [^{3}H]thymidine incorporation, was determined by liquid scintillation spectroscopy. To determine the Ia restriction of the T cells, I-A^d-specific MK-D6 and I-E^d-specific 14-4-4S antibodies were used as described (12). The requirement of antigenic processing of the peptides was tested by use of paraformaldehyde (13).

RESULTS AND DISCUSSION

Rationale for the Selection of Truncated Peptides. T cells obtained from mice primed *in vivo* with either intact 2F10 anti-id or HBsAg respond vigorously to *in vitro* stimulation with the 15-aa 2F10 peptide (9). We wanted to determine the minimal sequence of 2F10 peptide required to duplicate the responses (*in vitro* and *in vivo*) elicited by the intact 2F10 peptide and to establish which residues would constitute the B- and T-cell epitopes. Two separate and nonoverlapping regions from the HBsAg sequence have significant homology to the 2F10 peptide sequence (9). These two regions, S-(135–146) and S-(124–134), align with aa 1–12 and 5–15 of the 2F10 peptide, respectively (9). Peptide 1 (aa 1–12) and peptide 2 (aa 5–15) correspond to residues from the N-terminal and the C-terminal portions, respectively, of the 15-aa 2F10 peptide with an overlap from aa 5 to aa 12 (Fig. 1). These two truncated peptides would allow us to determine which of the two HBsAg sequences was relevant to the HBsAg and 2F10 peptide molecular mimicry.

The 15-aa 2F10 peptide sequence comprises complementary-determining region 3 (CDR3) and part of the adjacent framework region (FR3) of the 2F10 antibody (9). The next two truncated peptides, nos. 3 and 4, were designed so that peptide 3 represented the CDR portion of the 2F10 peptide, and peptide 4 represented its FR portion (Fig. 1).

Antibody Production by Truncated Variants of the 2F10 Peptide. Both the 2F10 peptide (uncoupled) and 2F10 peptide coupled to KLH elicit a significant antibody response against HBsAg (9). In BALB/c mice, i.p. injection with peptide 1, 2, or 3 coupled to KLH elicited anti-HBsAg antibodies, whereas injection with peptide 4 did not (Fig. 2). Thus in the case of 2F10 antibody, only its CDR portion has sequences that can mimic the nominal antigen (HBsAg) at the B-cell level. The profile and magnitude of the anti-HBs response elicited by peptide 1, 2, or 3 are unique to each peptide (Fig. 2). The magnitude of the antibody response obtained upon immunizing mice with the 15-aa 2F10 peptide is ≈10-fold higher than that obtained by immunizing mice with the truncated peptides. We believe that the differences in the extent and timing of anti-HBsAg antibody production elicited by these peptides are a function of the length of the peptide. It is possible that the FR sequence of 2F10 peptide might aid the CDR portion (B-cell epitope) in adopting a favorable conformation needed to elicit a more sustained antibody response. Similar results have been reported in other antigen systems, where increases in the length of a B-cell epitope or changes in residues flanking an epitope have resulted in increased antibody production (14, 15). Note that the presence of all or part of the CDR sequence was sufficient to result in anti-HBs production (i.e., immunization with peptide 1, 2, or 3). Further, since peptide 1 lacks the three C-terminal residues (SLY), it appears that their presence is not critical for the formation of the B-cell epitope.

Finally, as the anti-HBs response is T-cell dependent, it was important to determine whether the peptides could provide the required T-cell help for antibody production. The truncated peptides would provide T-cell help only if they contained functional T-cell epitopes, and we tested this by performing *in vitro* T-cell proliferation assays.

T-Cell Responses to the Truncated Peptides. BALB/c mice were primed *in vivo* with the 15-aa 2F10 peptide or with truncated peptides 1, 2, 3, or 4, and the primed T cells were tested *in vitro* for their ability to proliferate in response to the appropriate stimuli. 2F10 peptide-primed T cells responded *in vitro* to anti-id 2F10, HBsAg, S peptide, peptide 2, and peptide 3 (Table 1). It thus appears that the T-cell responses against the 15-aa 2F10 peptide are focused on the C-terminal portion of the peptide. This was further confirmed by the lack of stimulation of these cells by both peptides 1 and 4 (corresponding to N-terminal residues). Peptide 1 represents the sequence of 2F10 peptide without the C-terminal residues SLY. The failure of peptide 1 to stimulate 2F10 peptide-primed T cells indicates that peptide 1 either does not associate with class II major

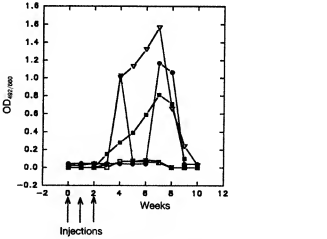

FIG. 2. Comparison of anti-HBsAg antibody responses elicited by immunization with 2F10 peptide (■), peptide 1 (●), peptide 2 (▽), peptide 3 (▼), or peptide 4 (□). Results are expressed as $OD_{492/690}$ of the average of five mice per experiment. Each curve is a representative of three (peptides 1 and 2) or two (2F10 peptide, peptides 3 and 4) experiments. Measurements were performed at either 1:2 dilution (peptides 1–4) or 1:20 dilution (2F10 peptide) of sera.

Table 1. *In vitro* proliferation of T cells primed with 2F10 peptide, truncated peptides, or HBsAg

Stimulus	Concentration, μg per well	[3H]Thymidine incorporation of cells elicited by peptide immunogens, cpm					
		2F10 peptide	Peptide 1	Peptide 2	Peptide 3	Peptide 4	HBsAg
2F10	50	49,607	84,698	90,873	131,897	156,462	50,986
2C3	50	3,326	2,971	2,826	1,773	1,024	170
HBsAg	0.5	24,551	2,649	4,008	103,082	1,174	104,719
2F10 peptide	0.5	46,630	64,582	160,387	35,860	131,979	174,916
Peptide 1	0.5	6,802	110,970	4,017	54,605	1,229	843
Peptide 2	0.5	29,954	3,270	160,724	67,808	981	63,630
Peptide 3	0.5	60,881	3,742	4,213	114,665	1,164	194,936
Peptide 4	0.5	3,895	5,277	4,932	1,797	169,191	445
S peptide	0.5	36,150	3,793	3,701	38,391	1,154	129,259
Control peptide	0.5	3,937	3,514	2,789	1,845	1,204	415
Medium	—	4,315	3,928	4,623	2,613	975	187

Results are expressed as the average cpm of [3H]thymidine incorporation of triplicate cultures of a representative experiment of either three (2F10 peptide, peptide 2, and peptide 4) or four (peptides 1 and 3) separate experiments.

histocompatibility molecules or that this sequence is not cross-reactive with the 15-aa sequence at the T-cell level. Peptide 1 is restricted by I-A^d molecules (unpublished data), thus its Ia contact residues are clearly not affected by the C-terminal truncation. Hence, it appears that the last three residues, SLY (aa 13–15), are important T-cell contact sites for 2F10 peptide-primed T cells.

To further understand the fine specificity of the T-cell response, we tested the *in vitro* proliferative response using T cells primed *in vivo* with peptide 3 or 4. Our rationale was that peptides 3 and 4 (representing two nonoverlapping regions of the 15-aa 2F10 peptide), when used as immunogens, would focus the T-cell responses only to these regions of the intact 2F10 peptide, allowing us to distinguish whether the sequences representing the CDR and/or FR of the 2F10 peptide could function as T-cell epitopes. In contrast to the very restricted *in vitro* proliferation pattern obtained with peptide 4-primed T cells, peptide 3-primed T cells showed substantial proliferation when stimulated *in vitro* by 2F10 anti-id, HBsAg, 2F10 peptide, S peptide, or peptide 1, 2, or 3 (Table 1). No response could be detected against peptide 4. That peptide 3 elicited T cells that crossreacted with S peptide and HBsAg argues strongly that this octapeptide is a functional T-cell epitope capable of eliciting T cells that can recognize HBsAg. Thus it appears likely that peptide 3 may function independently as a T-cell epitope and direct T-cell help necessary for the generation of the anti-HBs response. As peptide 3 was coupled to KLH, it is also possible that KLH-derived T-cell epitopes may also have provided T-cell help for anti-HBs production. That peptides 1 and 2 could induce *in vitro* proliferation of T cells generated *in vivo* as a consequence of priming with peptide 3 indicated that the fine specificity of T-cell response elicited by peptide 3 may be focused on the overlapping portion of peptides 1 and 2—i.e., the sequence CTRGYHGS of the 2F10 peptide. Further, as peptide 3 used as the immunogen in the above experiments lacks the residues CTR, it appears that GYHGS would be important contact sites for the T-cell antigen receptor.

T cells primed with peptide 4 responded *in vitro* only to peptide 4 itself, 2F10 antibody, and 2F10 peptide (Table 1). It was somewhat surprising that a small peptide such as peptide 4, a heptapeptide, was a functional T-cell epitope. That 2F10 peptide was recognized *in vitro* by the peptide 4-primed cells indicates that the N-terminal portion of 2F10 peptide may also function as a T-cell epitope. Peptide 4-primed T cells showed a complete absence of proliferation *in vitro* against HBsAg and S peptide. This indicates a total lack of crossreactivity at the T-cell level between peptide 4 and S peptide or HBsAg. This suggests that the FR sequence of the 15-aa peptide may not be involved directly in eliciting B- or T-cell responses that are HBsAg specific. T cells primed with peptide 1 or 2 were also restricted in their *in vitro* T-cell proliferation pattern (Table 1). Peptide 1-primed T cells proliferated only in response to stimulation with 2F10 anti-id, 2F10 peptide, and peptide 1. Similarly, immunization with peptide 2 elicited T cells that had *in vitro* reactivity only to 2F10 anti-id, 2F10 peptide, and peptide 2. No reactivity was observed against HBsAg and S peptide or peptide 3. This was surprising because there is crossreactivity between peptide 3 and S peptide and because the sequence of peptide 3 is contained within peptide 2. We considered the possibility that peptide 2 may be processed so that the resulting epitope may not have the peptide 3 sequence or may have only a part of it. Consequently, T cells primed *in vivo* to such a processed epitope of peptide 2 may not have any reactivity against peptide 3 or S peptide. Similarly, for peptide 1, the *in vitro* T-cell reactivity was limited to peptide 1, 2F10 anti-id, and 2F10 peptide. As T cells primed with peptide 1 showed no *in vitro* response against peptide 4 (Table 1), even though the sequence of peptide 4 is found in peptide 1. Again, it is possible that peptide 1 undergoes processing so that the resultant epitope may not have an intact peptide 4 sequence, thereby accounting for the absence of proliferation. Finally, T cells primed *in vivo* by HBsAg proliferate *in vitro* when stimulated with 2F10 antibody, HBsAg, 2F10 peptide, peptide 2, peptide 3, or S peptide. This further confirms the strong crossreactivity between the a determinant of HBsAg (S peptide) and peptides derived from the 2F10 antibody that contain the CDR sequence of the 2F10 anti-id.

Processing and Presentation of the Truncated Peptides. Most protein antigens undergo processing to small peptides which then bind to major histocompatibility molecules for successful presentation to a specific T cell. Both the anti-id 2F10 and HBsAg require processing to stimulate T cells (12). The 15-aa 2F10 peptide and peptides 3 and 4 functioned as T-cell epitopes in the present study. It was thus important to determine whether the 2F10 peptide did undergo processing, because this would suggest the possibility that pcptides 3 and 4 could be naturally produced, physiologically relevant T-cell epitopes. We were also interested in determining whether the smaller peptides (nos. 3 and 4) underwent further processing. We addressed the issue of the requirement of antigenic processing by using paraformaldehyde and chloroquine as inhibitors of antigen processing.

Our results (Table 2) indicate that 2F10 peptide, peptide 1, and peptide 2 needed processing before being presented to T cells. Peptides 3 and 4 (an octapeptide and a heptapeptide, respectively) did not require processing prior to antigen presentation. Identical results were observed with chloroquine (unpublished data). These results helped us to understand the results shown in Table 1. The inability of peptide 2-primed T cells to respond *in vitro* to peptide 3 and the total lack of response of peptide 1-primed T cells following stimulation *in*

Table 2. Effect on T-cell proliferation after treatment of antigen-presenting cells with paraformaldehyde

	[^{3}H]Thymidine incorporation, cpm			
	Peptide 3-primed T cells		Peptide 4-primed T cells	
Stimulus	No treatment	Paraformaldehyde	No treatment	Paraformaldehyde
2F10 peptide	126,860	131	164,986	638
Peptide 1	116,357	227	213	204
Peptide 2	81,562	339	228	195
Peptide 3	228,726	223,025	383	395
Peptide 4	287	204	225,186	184,926

In vitro proliferation of T cells (primed with either peptide 3 or peptide 4) was compared in the presence or absence of paraformaldehyde. Results are expressed as the average of [^{3}H]thymidine incorporation of triplicate cultures of a representative experiments of two separate experiments for each peptide.

vitro by peptide 4, strongly suggest that processing of peptides 1 and 2 may indeed influence their *in vitro* reactivity pattern.

Using MK-D6 (anti-I-A^d) and 14-4-4S (anti-I-E^d) mAbs we showed that 2F10 peptide and all its truncated variants were restricted by the I-A^d molecule (unpublished data). We have further confirmed this by demonstrating significant binding of radiolabeled peptide 3 with affinity purified I-A^d molecules (unpublished data). This observation supports our finding sequence alignment between VHAAHAE (I-A^d binding motif) and our four truncated peptides. VHAAHAE is a sequence which has been shown by Buus *et al.* (16) to be the core region of an ovalbumin peptide which binds to the I-A^d molecule. This region was later established as an I-A^d binding motif, since it showed significant sequence homology with many other I-A^d-binding peptides derived from other antigenic systems. The 2F10 pentadecapeptide and peptides 1, 2, and 3 had identical sequence alignments with VHAAHAE (Fig. 3*A*). Thus, there seems to be an anchor sequence within the 2F10 peptide which could be represented as GYHGS (aa 8–12). Peptide 4 lacks this anchor motif yet aligns with VHAAHAE (Fig. 3*B*) in another manner. We conclude that the 15-aa sequence of 2F10 peptide sequence has two functional T-cell epitopes represented by peptides 3 and 4, but only peptide 3 is crossreactive with the a determinant of HBsAg (S peptide) at both the B- and the T-cell level.

CONCLUSIONS

The 15-aa 2F10 peptide has been shown to contain a functional B-cell epitope and a T-cell epitope (9). The orientation and placement of cognate T- and B-cell epitopes are critical to elicit an antibody response (1). Hence, we were interested in determining the location of the T- and B-cell epitopes within the 2F10 peptide. Three possibilities existed: the T- and B-cell epitopes could be identical, adjacent to one another, or overlapping. To address this issue, we made truncated peptide sequences from within the pentadecapeptide. BALB/c mice were immunized with each truncated variant and their ability to elicit HBsAg-specific antibodies and T cells was tested. From our results we conclude that there are two functional T-cell epitopes in the sequence of 2F10 peptide, only one of which, represented by peptide 3, also functions as a B-cell epitope. Thus, T- and B-cell epitopes that mimic the a determinant of HBsAg are contained in a C-terminal octapeptide (peptide 3) sequence of the 2F10 peptide. The N-terminal portion of the 15-aa 2F10 peptide, represented by peptide 4, functions as a T-cell epitope but is unable to elicit T cells that are crossreactive with HBsAg. Thus, only the CDR portion of the 2F10 peptide sequence is responsible for the molecular mimicry. The reason that the 15-aa 2F10 peptide can elicit a stronger antibody response may be that the peptide 4 sequence (FR sequence) helps the peptide to attain an optimum conformation. Experiments involving peptides 1 and 2 helped us to establish that as long as all or a part of the CDR sequence was maintained, the peptides were capable of eliciting anti-HBs antibodies. This finding suggests that a short stretch, aa 8–12, has the important contact sites required to stimulate HBsAg-specific B cells.

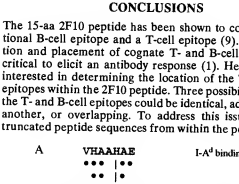

FIG. 3. Sequence alignment of I-A^d binding-motif sequence with amino acid sequences of 2F10 peptide and peptides 1–4. Alignment was done with the computer program GAP (21). Vertical bars indicate identity; dots indicate similarity.

An effective vaccine should contain two distinct immunological sites, one which promotes T-cell interaction (T_h epitope) and one which promotes B-cell interaction (B-cell epitope). Numerous reports have identified small peptide sequences which are capable of eliciting T_h-cell function. In contrast, there are very few examples of T-cell-stimulatory epitopes also representing B-cell epitopes. One such example is the hemagglutinin synthetic peptide [HA1-(177–199)] of the influenza hemagglutinin system (17). The 2F10 peptide and now peptide 3 of our anti-id in the HBsAg system are additional examples. HA1-(177–199) contains three overlapping T-cell epitopes and a B-cell epitope. Two of its T-cell epitopes are restricted by I-A^d and one by I-E^d (18). This finding is in contrast to our observations with the 2F10 peptide. The 2F10 peptide contains a B-cell epitope that also functions as a T-cell epitope and has another, nonoverlapping T-cell epitope. Both T-cell epitopes are restricted by I-A^d. To the best of our knowledge, peptide 3, an octapeptide, is the smallest sequence identified which contains both a B- and a T-cell epitope. Our T-cell proliferation assay results make it clear that 2F10 peptide has to undergo processing for T-cell recognition. A search for sequence motifs of cleavage sites for enzymes involved in antigenic processing, indicated the bond between Arg^7 and Glu^8 as a putative site for enzymatic cleavage. The possible candidate processing enzyme would be an endosomal/lysosomal trypsin-like (EC 3.4.21.4) serine protease or a cathepsin B-like (EC 3.4.22.1) thiol protease which would preferentially cleave on the C-terminal side of arginine. These enzymes are implicated in the antigenic processing of myoglobin (19).

To assume that the above-mentioned cleavage point is the only site within our sequence where processing occurs would

not permit the complete understanding of all our data. For example, T cells primed *in vivo* with peptide 2 did not show a proliferative response against peptide 3. If peptide 2 were cleaved between Arg^7 and Gly^8, it would give rise to the peptide 3 sequence *in vivo*. Therefore, the T cells primed *in vivo* against this processed peptide (peptide 3 sequence) should recognize peptide 3 *in vitro*. As the results indicate otherwise, we believe that the loss of N- and C-terminal residues of 2F10 peptide in the sequences of peptides 1 and 2, respectively, may alter the cleavage site so that peptides 1 and 2 get processed at a different site. Results obtained with these truncated synthetic peptides further stress the effect that sequences flanking the minimal immunodominant epitope may exert on the nature of antigenic processing and, consequently, on T-cell recognition. Similar observations have been made by other investigators (20). Further studies using specific protease inhibitors may help elucidate the nature of processing of the 2F10 peptide, peptide 1, and peptide 2. In conclusion, these results establish that (*i*) a 15-aa peptide can contain two separate T-cell epitopes stimulating distinct responses; (*ii*) an octapeptide can function as both a B- and a T-cell epitope and elicit responses which are specific to the native protein antigen; (*iii*) though the N-terminal portion of the 15-aa 2F10 peptide functions as a T-cell epitope, it is unable to elicit antigen-specific T cells and therefore does not contribute to the mimicry of 2F10 at the T-cell level; and (*iv*) even relatively short antigenic peptides (2F10 peptide and peptides 1 and 2) can be processed further before binding to Ia molecules.

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